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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : A61B 5/00, G01N 33/48

A1

(11) International Publication Number: WO 89/ 01758

(43) International Publication Date: 9 March 1989 (09.03.89)

(21) International Application Number: PCT/US88/03027

(22) International Filing Date: 1 September 1988 (01.09.88)

(31) Priority Application Number: 093,482

(32) Priority Date: 4 September 1987 (04.09.87)

(33) Priority Country: US

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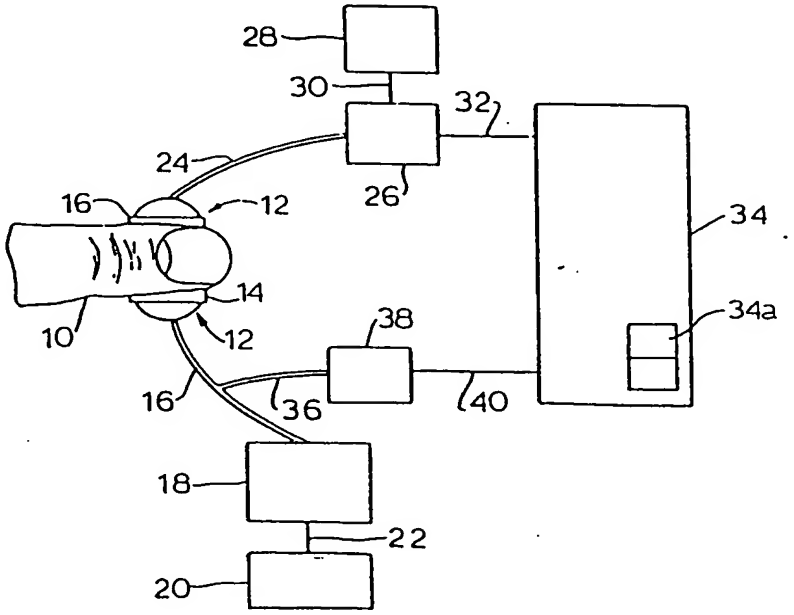
(81) Designated States: AT (European patent), AU, BE (European patent), BR, CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU.

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVELY DETERMINING THE CONCENTRATION OF A DILUTE COMPONENT IN A LIGHT- OR OTHER RADIATION-SCATTERING ENVIRONMENT



(57) Abstract

A spectrophotometric method is described of quantitatively determining the concentration of a dilute component in either a clear or a strongly light-scattering environment containing same in unknown concentration together with a reference component of known concentration, by a series of contemporaneous radiation-directing (14) and measurement steps (16) of radiation of selected varying wavelengths. Specific applications are disclosed involving the *in situ, in vivo,* (Figure 7) non-invasive spectrophotometric determination of blood-borne as well as tissue species, e.g., hemoglobin, and oxyhemoglobin, and intra-cellular enzyme cytochrome *c* oxidase, in human body parts such as fingers, (Figure 7), hands, toes, feet, earlobes, etc., as well as organs such as the brain (Figure 8), skeletal muscle, liver,

SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVELY DETERMINING THE CONCENTRATION OF A DILUTE COMPONENT IN A LIGHT- OR OTHER RADIATION-SCATTERING ENVIRONMENT

BACKGROUND OF THE INVENTION

Field Of The Invention

This invention generally relates to a spectrophotometric method of quantitatively determining the concentration of a dilute component in a light- or other radiation-scattering environment containing the dilute component in combination with a reference component of known concentration.

Description Of The Related Art

In many fields of technology there is a need for quantitative determination of dilute component concentrations in environments where the dilute component is in combination with a reference component of known concentration. Examples of illustrative environments of such type include enzymes, proteins, and metabolites in corporeal fluids; acidic fumes or gaseous components (e.g., hydrogen sulfide and sulfuric acid, nitric acid, carbon monoxide, etc.) in the atmosphere; salt concentrations in sea water undergoing desalination; ozone in ozone-enriched air utilized in waste water ozonation systems, etc.

In particular, there has been a specific need in the medical and health care fields for a non-invasive, continuous, atraumatic, in vivo, in situ determination of amounts of critical metabolic indicators in body fluids or tissues of human patients. Examples of such body fluids include the blood and fluids associated with the lymphatic and neurological systems of the body. Further specific examples involving the human circulatory system include the monitoring of glucose and of oxygenated/de-oxygenated, arterial/venous colored hemoglobin in the blood stream. In addition, monitoring in localized tissue, such as brain and muscle, of certain enzyme species such as the cytochrome c oxidase enzyme (unofficially better known as cytochrome a,a3) or metabolic substrates (such as glucose) or products (such as carbon

even though the influent radiation is penetrative of the body elements of the corporeal system, e.g., bones, musculature, organs, and the like, since the scattering of radiation during its passage through the corporeal system is extensive and highly variable in character. Such scattering not only adds an unknown loss of radiation to the required information regarding specific absorption but by multiple scattering it also lengthens to an unknown degree the path length of those photons eventually emerging form the body element. As a result, it has not been possible to determine in an in vivo situation what the effective path length, d, of the impinged radiation actually is, prior to measurement of the transmitted or reflected radiation derived therefrom. In consequence, the absolute quantitation of solute concentrations in corporeal systems has been severely adversely limited.

Faced with the alternatives of invasive and traumatic sampling of the corporeal fluids of interest, or spectrophotometric methods which realize only qualitative or at best semi-quantitative measurement of changes in tissue or body fluid solute concentrations, there is a substantial perceived need in the art for a non-invasive, in vivo method of quantitatively determining the concentration in corporeal solution of a solute in a body fluid solvent.

A similar need exists in numerous other fields in which absolute concentrations of dilute component species in fluid media would materially assist the characterization of the fluid system. An example is atmospheric monitoring of "acid rain," i.e., airborne acidic contaminants which have in recent years proliferated and been determined to cause widespread biospheric damage, including the defoliation of forest stocks and spoliation of natural bodies of water and other aqueous environments. It is anticipated that in coming years with the increasing severity of the acid rain problem, correspondingly greater scientific and legislative efforts will be focused on the monitoring of acid rain with a view to controlling and

output signal representing the difference in or ratio of absorption of the measuring and reference wavelengths by the organ or other corporeal portion of the body as a function of the state of the metabolic activity in vivo, which may be converted to a signal providing a substantially continuous measure of such activity. A related spectrophotometric reflectance technique is disclosed in U.S. Patent 4,223,680 to F. F. Jobsis.

U. S. Patent 4,655,225 to C. Dahne et al discloses a spectrophotometric system for non-invasive determination of glucose concentration in body tissue. The system involves irradiation of the exterior body portion with an optical light whose transmittance or reflectance is collected at selected band wavelength values for the glucose absorption spectrum and at a selected band wavelength value for the absorption spectrum of background tissue containing no or insignificant amounts of glucose. The measuring and reference radiation collected is then converted into electrical signals and utilized to determine glucose concentrations.

It is an object of the present invention to provide an improved method and apparatus for indirectly quantitatively, spectrophotometrically determining the amounts of a dilute component by using a reference component in the environment of interest.

It is another object of the invention to provide such a method for non-invasive, in vivo quantitative determination of the concentration of a dilute solute component in a corporeal solvent environment.

Other objects and advantages of the present invention will be more fully apparent from the ensuing disclosure and appended claims.

determined similarly at other near-by wavelength(s),may be employed to calculate the concentration of the dilute component relative to the reference component.

In a system in which the reference component is present in known concentration, the apparent pathlength may be determined by absorption measurements taken in the environment of unknown pathlength in the same electromagnetic spectral region. The difference between the resulting absorption values is calculated as the differential absorbance in the environment whose pathlength is to be determined. The tabulated or previously determined extinction coefficient values of the pure reference component at such wavelengths are then employed to calculate the differential extinction coefficient, as the difference between the respective extinction coefficient values. When the differential absorbance is then divided by the differential extinction coefficient, the result is the apparent effective pathlength of the environment. When the electromagnetic radiation emitter and detector spacing distance is measured, the pathlengthening factor for the system is determined as the ratio of the apparent effective pathlength to the actual emitter-to-detector spacing distance.

In another selected, specific aspect, the invention relates to a spectrophotometric method of quantitatively determining the concentration of a dilute component in an environment containing the dilute component of known identity but of unknown concentration in combination with a reference component of known concentration, by a series of successive, substantially contemporaneous measurements of transmitted and/or reflected radiation at selected wavelengths, comprising:

(a) determining the apparent effective pathlength in said environment;

A further aspect of the invention relates to a spectrophotometric method of quantitatively determining the concentration of a dilute component in an environment containing the dilute component of known identity but of unknown concentration in combination with a reference component of known concentration, comprising:

(a) directing at the environment incident electromagnetic radiation at a number of wavelengths in a selected spectral region at which the dilute and/or reference components exhibit absorption for the electromagnetic radiation, the number of such wavelengths being determined by the number of dilute and reference components in the environment, and the scattering characteristics of the environment;

(b) determining the absorbance by the environment of the electromagnetic radiation at the various wavelengths and the relative intensities of the absorption contributions of the dilute and reference components and scattering losses from the environment at each of such wavelengths;

(c) at each of such wavelengths, establishing absorption equations of the form:

$$Abs_W = \sum_{i=1}^{n} x_i A_i + zR + S,$$

wherein: Abs_W is the absorbance by the environment, containing the dilute and reference components, of the incident electromagnetic radiation of wavelength w; x_i is the relative intensity of the absorption contribution of the associated dilute component A_i, and wherein terms of the form $x_i A_i$ are set forth for each of the dilute components; n is the number of dilute components; z is the relative intensity of the

Still another aspect of the invention relates to apparatus for spectrophotometrically quantitatively determining the concentration of a dilute component in an environment containing the dilute component of known identity but of unknown concentration in combination with a reference component of known concentration, comprising:

(a) means for producing electromagnetic radiation of known wavelengths and directing said radiation into the environment to be characterized for the dilute component;

(b) means for detecting electromagnetic radiation emanating from and/or reflected from the environment and producing therefrom an electrical signal corresponding thereto;

(c) means for receiving said electrical signal and producing therefrom electrical signals at corresponding to said different wavelengths;

(d) means receiving and operatively responsive to said electrical signals corresponding to said different wavelengths, to establish absorbance equations responsive to said electrical signals corresponding to said different wavelengths, wherein absorbance at each of the wavelengths is expressed as a function of the relative intensities of the absorption contributions of the dilute and reference components and the concentrations of the dilute and reference components, and for calculating the amounts of the absorbing species by solution of said absorbance equations; and

(e) means for displaying the calculated concentrations of said dilute and reference components.

In general, the number of wavelengths employed for concentration determination of the dilute component(s) in the system will be equal to the number of absorbing species (i.e., the number of dilute component(s) and the reference

A second preferred application using a transillumination mode is the measurement of hemoglobin content in blood by the quantitative determination of the hemoglobin and water content of the pulsatile increases in blood content of a finger or earlobe as it pulsates with each heartbeat. Measurements can similarly be made of other blood-borne constituents (e.g. glucose, lipids, cholesterol, carbon dioxide, etc.) that possess absorptive characteristics in the invisible, infrared or other parts of the electromagnetic spectrum.

In other general applications of the present invention, quantitative determinations can be made in light- or other radiation-scattering media, mixtures, or solutions, of any and all constituents possessing absorption characteristics in a spectral range in which a reference compound of known concentration also possesses significant absorption characteristics. Such applications are not limited to the visible or near infrared parts of the spectrum but can be performed in any part of the electromagnetic spectrum in which absorption of the radiation in either a transillumination or in a reflective mode is not so intense as to preclude the acquisition of a radiation signal strong enough for routine instrumental analysis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a plot of the absorption spectrum of pure component water.

Figure 2 is a plot of the absorption spectrum of water in an environment exhibiting a flat baseline, B, associated with radiation scattering.

Figure 3 is a plot of the absorption spectra of water, hemoglobin, and oxyhemoglobin, with a first baseline attributable to radiation scattering by the environment.

liquid, a solid, or a gaseous mixture, in which multiple scattering lengthens the geometrically measurable optical pathlength. The present invention overcomes this problem of undefined pathlength in media which have light-absorbing properties of their own in the electromagnetic radiation spectral region of the wavelength range in which the material whose concentration is to be determined is radiation absorptive.

The general broad applicability of the invention will be clear from the ensuing description of the invention, even though this description utilizes the near infrared spectral range in the illustrative examples hereinafter set forth. It should also be noted that in a spectral range (e.g., the visible range) in which the solution or any other known component does not absorb the impinged radiation, an indicator may in some instances be desirably added (specifically in in vitro determinations) to the solution to establish the effective pathlength traversed by the photons.

The Beer-Lambert law defines the basis of the spectrophotometric determinations of concentrations of radiation absorbing materials. It emphasizes that the absorption of light (or other radiation) depends on just two conditions: the efficiency of the molecules or atoms to absorb light and the number of such molecules or atoms in the light path. Two aspects should immediately be noted. The efficiency of radiation absorption varies at different wavelengths making it necessary to use a narrow "monochromatic" band of light and to state the efficiency with which the material absorbs that light. This parameter is called the extinction coefficient. The other aspect is the consequence that when the length of the light path through the selection or mixture is known, the only variable determining the number of molecules or atoms in the solution or mixture is the concentration. The entire field of quantitative analysis using bench-top spectrophotometers applies this fact by using

The extinction coefficient is standardly given in the form of the amount of absorption produced over a 1 cm pathlength by a 1 molar solution (one molecular weight of solute contained in one liter of solution). Values for molar extinction coefficients are commonly available in published tables and are usually given for wavelengths of maximal absorption.

Transillumination of a material with intense light-scattering properties results in a significant fraction of photons falling on the detector having had a tortuous pathway that increased the distance traversed beyond the direct geometric length of the sample. In the most extreme mode, viz., reflectance spectrophotmetry, absorbance spectra are taken utilizing the photons scattered out of the sample, either obliquely (typically 90° angle observation) or back-scattered (same surface observation). Not only is the pathlength then unknown it is undefined especially in the case of same surface observations. The mean depth of penetration before back scattering occurs is difficult or impossible to determine. In either case the effective pathlength is unknown and concentrations can not be determined by the Beer-Lambert law.

The present invention substitutes a totally new approach to determine concentrations in various media - solutions, gas mixtures, and solids - an approach not predicated on pathlength but on simultaneous measurement of the amount of medium traversed. In solutions this method provides therefore a statement concerning the amount of solvent encountered. From this parameter and the strength of absorption by the solute, the concentration of the solution can be derived. A prerequisite is that the absorption bands occur at relatively closely-spaced wavelengths, scattering being wavelength dependent, especially in the visible and ultraviolet wavelengths regions of the spectrum.

per liter of solution which means

$$\frac{\text{grams}}{\text{molecular weight}} \quad \text{per liter.}$$

Since the weight of a liter of water is 1000 grams and its molecular weight is 18, pure water exists in the form of of 1000/18 = 55.6 molar "solution." Thus water is an indicator present at known concentration. However, in terms of the present discussion it is clarifying to note, for the special case of water or any other spectrophotometrically measurable solvent, that if it is known that a 1 cm cuvette filled with pure water shows an Absorbance of say 0.50 at a given wavelength, then an Absorbance value of 6.00 found at that same wavelength over an unknown pathlength shows that that pathlength must have been 12 cm.

In this discussion, it may also be noted that the solute molecules do displace water molecules to a certain extent, lowering thereby its concentration in the solution as compared to its own concentration in pure water. This effect is, however, very small for the dilute solutions encountered in most situations. For example, the most concentrated salt component of blood (NaCl) produces a less than 2.6 ml increase in volume when dissolved in a liter of water. Therefore, the water content of the resulting "physiological salt solution" is decreased by less than 0.26%. The errors thus created are far smaller than many uncertainties inherent in this or any other spectrophotometric methodology.

The parallel argument for the macro-molecular and so-called "formed" components of tissues, however, is best reduced to terms of water content. Typically, for soft tissues the water content is 85% percent. A correction of about 15 to 20% is significant and could be applied in such cases. In that case the determined concentrations would be in terms of total tissue mass. This may, however, not be preferable to an expression in terms of total tissue water

in the observed Absorbance values at these respective wavelengths is a measure of either the amount of water encountered by the photon stream along their optical path, or in the case of a dilute solution (i.e., a solution in which the water concentration remains approximately 55.6 molar) a measure of the pathlength. Therefore, if the actual differential Absorbance, i.e., the difference in absorption values at the wavelength values of 980 and 1100, nanometers is determined, the effective pathlength of the test system can be derived by dividing the measured differential absorbance by the differential absorbance value between 980 and 1100 nanometers for 1 cm of water.

For example, Figure 2 shows the absorption spectrum for water (curve A), over the spectral range of from about 900 to about 1100 nanometers, with a flat baseline attributable to environmental scattering (baseline B). The plot shows the peak of the absorption spectrum for water (curve A) at a first wavelength, λ_1, and a trough in the spectrum at a second wavelength λ_2. The difference in absorption for water at the respective λ_1 and λ_2 values is indicated by the quantity $A_1 - A_2$, as the differential absorption in an environment exhibiting background scatter producing a flat baseline. The practice of subtracting absorption signals at adjacent wavelength values in this manner, where the known component differs significantly in its absorbance at the respective wavelengths, amounts to subtracting an existing wavelength-independent baseline of loss by scattering. The intensity of the remaining A_1-A_2 value provides a measure of the effective pathlength. What has not yet been discussed is the determination of the concentration of other absorbing components dissolved in the water and their effect on the water measurement.

are denoted by the letters A, C, D, and B respectively, and wherein the baseline indicates scattering of a curvilinear wavelength-dependent character, a number of extra wavelengths will be required to correct for the curvature of the baseline. The higher the degree of accuracy required for the calculated concentrations, the greater the number of wavelength determinations that must be employed.

METHODOLOGY

The method of the present invention has particular applicability to the determination of concentrations of blood components, such as the aforementioned hemoglobin and oxyhemoglobin, in body extremities where transillumination is employed, i.e., a source of radiation is impinged on the body part and collected at another exterior region of such body part. This methodology is applicable to body parts such as fingers, toes, earlobes, and other organs up to and including infants' heads. Alternatively, reflectance spectrophotometry may be employed in portions of the body where transillumination is impractical due to the mass and optical density of the body part involved, e.g., the adult head, lungs, kidneys, etc.

The spectra of water (curve 1), hemoglobin (curve 2), and oxyhemoglobin (curve 3) are shown in Figure 6 in the near infrared spectrum, over the range from about 700 to 1400 nanometers. In addition, the absorption curve of cytochrome $\underline{a}, \underline{a}_3$ is illustrated for later discussion. These spectra were obtained by bench-top spectrophotometry using transillumination. The water spectrum is a so-called absolute spectrum, i.e., obtained from a cuvette full of water using an empty cuvette as a "blank" to determine I_o at each wave length. The other spectra are of the hemoglobin and oxyhemoglobin compounds each dissolved in watery solution against a water blank.

Considering now the 900 to 1400 nm region of the near infrared spectral range, it is noted that the contributions of hemoglobin become negligible beyond 1150 nm approximately. Thus, the effective optical pathlength through a very small body part such as a finger can be determined by measuring at the trough, at 1270 nm, and at either adjacent peak, i.e. at approximately 1200 or 1400 nm. By subtracting the Absorbance values at the two wavelengths from each other, the differential absorption value is found. When such differential absorption value is divided by the differential absorption (extinction) coefficient for 1 cm of water, the apparent effective pathlength is determined. Assuming an equally flat scattering effect in the adjacent 700 to 900 nm region and with the knowledge that the finger tissues do not contain a measurable amount of cytochrome a,a_3 or other species absorbing in this range, it is possible to calculate the exact amounts of the two hemoglobins by transilluminating with any two wavelengths in the 700 to 900 nm range and using the pathlengthening factor established above.

This most simple case is often complicated by a number of factors. In the case of transillumination of a baby's head, for example, the thickness of the baby's head, makes it impractical to use a wavelength such as 1400 nm at which the intensity of the water absorption results in so much light loss that the remaining signal becomes difficult to detect. In this case four wavelengths are chosen in the 900 to 1100 nm range and the absorption intensities are measured. From previous experiments the contributions to the extinction by each absorbing species plus that by the light scattering have been established using approximate models. Best suited for the latter are the corresponding body parts from corpses or appropriate animal models. These may be perfused alternately with hemoglobin-free solution, with oxygen-free blood (for the Hb contribution), and with fully oxygenated

The above described methodology applicable to hemoglobin and oxyhemoglobin concentrations, may be generalized and broadly stated as a spectrophotometric method for quantitatively determining the concentration of a dilute component in an environment containing the dilute component of known identity but of unknown concentration in combination with a reference component of known concentration, in which the following steps are carried out:

(a) directing at the environment incident electromagnetic radiation at a number of wavelengths in a selected spectral region at which the dilute and/or reference components exhibit absorption for the electromagnetic radiation, the number of such wavelengths being determined by the number of dilute and reference components in the environment, and the scattering characteristics of the environment;

(b) determining the absorbance by the environment of the electromagnetic radiation at the various wavelengths and the relative intensities of the absorption contributions of the dilute and reference components and scattering losses from the environment at each of such wavelengths;

(c) at each of the aforementioned wavelengths, establishing absorption equations of the form:

$$Abs_w = \sum_{i=1}^{n} x_i A_i + xR + S$$

wherein: Abs_w is the absorbance by the environment, containing the dilute and reference components, of the incident electromagnetic radiation of wavelength w; x_i is the relative intensity of the absorption contribution of the associated dilute component A_i, and wherein terms of the form $x_i A_i$ are set forth for each of the dilute components; n is the number of dilute components; z is the relative intensity of the absorption contribution of the reference component; R is the

In pulse oximetry, the color of the extra blood that swells the finger with each pulse is determined, i.e., the relative amounts of Hb and HbO_2, thus providing a measure of the degree of oxygen saturation of the blood. This technique does not provide information on the total amount of hemoglobin in the blood. Adding a measure of the increase of water with each pulse can be accomplished by using an H_2O absorption signal to measure each pulsatile increase in blood volume in the finger. In this way the actual hemoglobin concentration in the blood can be calculated. The value of this number is general, and not limited to the specific organ (such as the finger) from which it was derived. The hemoglobin content thus determined provides important diagnostic information for such conditions as anemia or polycythemia. In addition, the hemoglobin content is required to determine the actual oxygen content of the blood since most of it is carried combined with hemoglobin in the form of oxyhemoglobin. With the hemoglobin content known and the percent of O_2-saturation of the hemoglobin obtained by standard oximetry the much more significant O_2-content parameter can be calculated quite simply.

In other organs of larger diameter, e.g. the head, limb musculature, etc., transillumination can be performed as long as the thickness of the tissue does not preclude the acquisition of an instrumentally useful signal after the transmitted radiation has passed through the tissue. In this respect it is to be noted that one cm of water absorbs approximately 80% of the 1400 nm near infrared radiation beamed throught it. Transillumination of an infant's head of 5 cm diameter would show an extinction of approximately 99.97% of the incident near infrared photons by absorption alone. In turbid samples, however, this loss can be increased and overshadowed by losses due to scattering away from the detector and by additional absorption attributable to pathlengthening produced by the multiple scattering encountered by the photons eventually arriving at the detector. Although these light losses are very severe, useful

component". It should be noted parenthetically that in cases of edema a shift of water from blood and lymphspaces into the cells takes place. In the brain, due to the nonelastic nature of the cranium which forms a pratically closed system, cerebral edema leads to increased intracranial pressure and consequently a forcing out of meningeal fluid and blood. However total intracranial water content remains the same. Incidentally, the loss of hemoglobin compared to the total water signal constitutes an excellent noninvasive indication of intracranial pressure build-up, a potentially fatal affliction.

In the preceding example, absorption by the main oxygen utilizing exzyme cytochrome c oxidase ("cytochrome a,a_3" or cyt a,a_3") was ignored. In view of this enzyme's relatively small contribution to the overall spectrum the resulting error produced in the hemoglobin data is negligible. In the event, however, that cyt a,a_3 information is desired, two more appropriately spaced wavelengths are required, one in the 825 nm region and the other in the 865 nm region. Although a water band does not exist in that exact region the 980 nm peak is relatively near, and hemoglobin which absorbs in both regions can be used as a bridging reference to determine the enzyme concentration.

PREFERRED EMBODIMENTS

Before describing the apparatus that can be utilized to make the measurements referred to above, three caveats should be added to the general principles used in the above example.

The first is the fact that a narrow banded, i.e., relatively monochromatic, light source is an important advantage in constructing incisive algorithms. It is quite clear from Fig. 6 that a photon source providing a narrow band of light, say 5 nm width, will produce much less overlap between absorption characteristics than a broad one, say with a 50 nm spread of wavelengths among its photons.

Figure 7 is a schematic diagram of a spectrophotmetric system for quantitatively determining the concentration of blood dilute components in a human finger with reference to water contained in the finger.

As previously alluded to, the amount of water encountered by the photons in a body part must be established first. This can be done either by measurements in the spectral range in which water is pratically the only absoring species or by multiwavelength differential spectrophotometry if other absorbing species are present. In the human finger water may best be determined by suitable spectrophotometric determinations on fingers of corpses. If such measurements must be made in a region of absorption band overlap with hemoglobin the blood must be replaced by a suitable non-absorbing scattering fluid to mimick the scattering by the red blood cells. Examples of suitable scattering fluids include fluorocarbon blood substitute solutions, calcium carbonate suspensions in saline solution, etc. In such "bloodless" systems, the spectrophotometric characteristics of the corpse fingers may be determined against pure water as a reference standard, to determine the apparent effective optical pathlength for radiation in a given spectral region, as passed through the finger to effect transillumination thereof. By numerous determinations of such type, a database of optical lengths for various types of human fingers (e.g., baby, adolescent, adult; Black, Caucasian, Oriental; etc.) may be developed. In this respect, it is to be noted that melanin is a pigmentation species which is present in varying degrees depending on the race and origins of the human subject. It in some instances may be desirable to treat melanin or other pigmentation-related agents as additional absorbing species in the system and to add further radiation-directing and measurement steps of additional wavelength(s) in determining the concentration of the desired dilute component in the corporeal system under study. Alternatively, routines for data acquisition and alogrithms calculation can be incorporated as software in a microprocessor-based system to

As applied to the apparatus shown in Figure 7, the finger 10 has mounted thereon two "optrode" assemblies 12, comprising a source optrode 14 and a collection optrode 16. The direction of transillumination is immaterial: a path through the finger nail may be preferable in certain instances.

The source optrode 14 is connected via optical fiber cable 16 to a light source 18, which in this illustrative embodiment comprises multiple solid state lasers energized by power supply 20 via power supply feedline 22. The laser source 18 emits electromagnetic radiations in the near infrared region, each of a monochromatic character, which are transmitted by the fiber optic cable 16 to the optrode 14 for impingement on the associated surface of finger 10, and transillumination thereof.

The resulting transmitted electromagnetic radiation is collected by the detector optrode 16 and passed via fiber optic cable 24 to an appropriate transducer 26 which in this illustrative embodiment comprises a photomultiplier tube energized by high voltage power supply 28 via power supply feedline 30. The sensed transilluminated signal passing from optical fiber cable 24 to the photomultiplier tube is amplified therein and passed by signal transfer means 32 to the signal processing module 34. In the event that a low voltage powered, "solid state" detector of small size is employed, the detector can be incorporated in the detector optrode. Fiber optic cable 24 is then replaced by an electrical cable directly to the subject.

As shown, the fiber optic cable 16 contains a small separate bundle branch line 36 which transmits a fraction of the monochromatic light from laser source 18 which is directly scattered back by the skin. It is coupled by cable branch line 36 to a photodiode 38, which transmits an electrical signal in signal wire 40 to the calculation module 34, which may for example comprise a digital electronic computer or may comprise a dedicated microprocessor unit or units.

In the Figure 8 system, incident electromagnetic radiation is emitted from optrode 114 and provides photons capable of penetrating both the skin and bone layer as well as the gray matter and white matter of the subject's head. Those photons which are reflected to the optrode 116 are sensed and the resulting detection signal is transmitted by fiber optic cable 124 to the photodetection and calculation module components, as previously described in connection with Figure 7.

Although the invention has been described with primary reference to the detection and determination of concentrations of dilute components such as tissue components and blood-borne species in body parts (whole tissue/whole organ environments) such as fingers, hands, toes, feet, earlobes, heads, and the like, using near infrared radiation, it will be apparent that the applicablity of the invention is not so limited. The method of the invention may be applied to the determination of any dilute component in an environment containing a reference component of known concentration and in any range of the electromagnetic spectrum in which spectrophotometric absorbance techniques can be practiced.

Illustrative examples of such alternative applications include but are not limited to, the measurement of acid rain constituents, carbon monoxide, or other air pollution species in atmospheric and oceanic/riparian environments; and the detection of toxic gas species in semiconductor manufacturing operations and industrial gas purification processes.

Further, while the invention has been shown and described with reference to illustrative embodiments, it will be apparent that other variations, modifications and embodiments are possible, and all such apparent variations, modifications and embodiments are to be regarded as being with the spirit and scope of the present invention.

THE CLAIMS

What is Claimed is:

1. A spectrophotometric method of quantitatively determining the concentration of a dilute component in an environment containing the dilute component of known identity but of unknown concentration in combination with a reference component of known concentration, by a series of successive, substantially contemporaneous measurements of transmitted and/or reflected radiation at selected wavelengths, comprising:

(a) determining the apparent effective pathlength in said environment;

(b) directing at said environment incident electromagnetic radiation of a first wavelength in a selected spectral region at which the dilute and/or reference component(s) exhibit absorption for the electromagnetic radiation;

(c) measuring the first wavelength radiation transmitted and/or reflected by the environment;

(d) directing at the environment incident electromagnetic radiation of at least one other wavelength in the selected spectral region at which the dilute and/or reference component(s) exhibit absorption of different relative intensities than for the first wavelength incident radiation;

(e) measuring the other wavelength radiation transmitted and/or reflected by the environment;

(f) determining extinction coefficient values for the dilute component at said first and other wavelengths in said environment; and

4. A method according to claim 1, wherein a second dilute component of unknown concentration is contained in said environment, and wherein electromagnetic radiation of a third wavelength in said selected spectral region is directed at said environment at which said second dilute component exhibits an absorption for the electromagnetic radiation, and the third wavelength radiation transmitted and/or reflected by the environment is measured, and employed to determine the relative amount of the second dilute component to the amount of the reference component.

5. A method according to claim 4, wherein three simultaneous modified Beer-Lambert equations are established for the concentration of the dilute components and reference component in said environment.

6. A method according to claim 1, wherein said environment effects scattering of said electromagnetic radiation which is independent of wavelength of said radiation, and wherein the transmitted and/or reflected radiation is measured at a single additional wavelength.

7. A method according to claim 1, wherein said environment effects scattering of said electromagnetic radiation to an extent which is of sloped linear relationship to wavelength, and wherein the transmitted and/or reflected radiation is measured at an additional two wavelengths.

8. A method according to claim 1, wherein said environment effects wavelength scattering of said electromagnetic radiation which is a non-linear function of wavelength, and wherein the transmitted and/or reflected radiation is measured at an additional at least three wavelengths.

9. A method according to claim 1, wherein said environment is a corporeal environment.

concentration is thus determined, as a reference component for the second dilute component in said second spectral region.

17. A method according to claim 9, wherein said corporeal environment comprises a body portion selected from the group consisting of heads, fingers, hands, toes, feet, and earlobes.

18. A method according to claim 1, wherein said electromagnetic radiation is infrared radiation having a wavelength in the range of from about 700 to about 1400 nanometers.

19. A method according to claim 9, wherein said reference component is water.

20. A spectrophotometric method of quantitatively determining the concentration of a dilute component in an environment containing the dilute component of known identity but of unknown concentration in combination with a reference component of known concentration, comprising:

(a) directing at the environment incident electromagnetic radiation at a number of wavelengths in a selected spectral region at which the dilute and/or reference components exhibit absorption for the electromagnetic radiation, the number of said wavelengths being determined by the number of dilute and reference components in the environment, and the scattering characteristics of the environment;

(b) determining the absorbance by the environment of the electromagnetic radiation at the various wavelengths and the relative intensities of the absorption contributions of the dilute and reference componets and scattering losses from the environment at each of said wavelengths;

but of unknown concentration in combination with a reference component of known concentration, comprising:

(a) means for producing electromagnetic radiation of known wavelengths and directing said radiation into the environment to be characterized for the dilute component;

(b) means for detecting electromagnetic radiation emanating from and/or reflected from the environment and producing therefrom an electrical signal corresponding thereto;

(c) means for receiving said electrical signal and producing therefrom electrical signals corresponding to said different wavelengths;

(d) means receiving and operatively responsive to said electrical signals corresponding to said different wavelengths, to establish absorbance equations responsive to said electrical signals corresponding to said different wavelengths, wherein absorbance at each of the wavelengths is expressed as a function of the relative intensities of the absorption contributions of the dilute and reference components and the concentrations of the dilute and reference components, and for calculating the amounts of the dilute and reference components by solution of said absorbance equations; and

(e) means for displaying the calculated concentrations of said dilute and reference components.

22. A method according to claim 1, wherein subsequent to determination of the concentration of the dilute component in the environment, the environment is monitored for changes in said concentration.

23. A method according to claim 20, wherein subsequent to determination of the concentration of each of the dilute and reference components in the environment, the environment is monitored to determine changes in said concentrations.

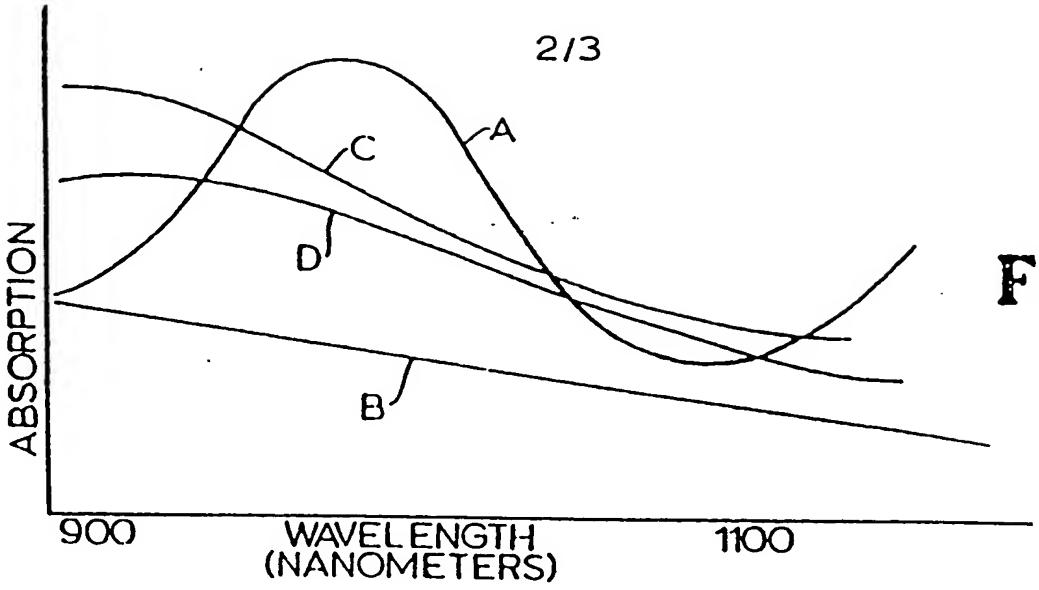


FIG. 4

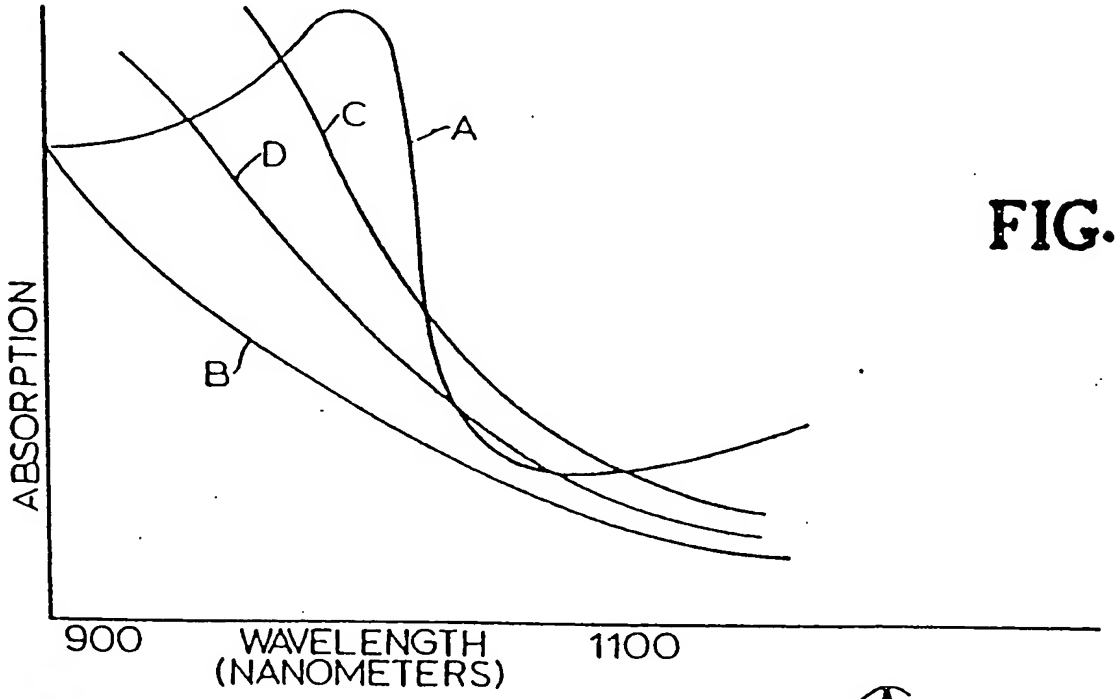


FIG. 5

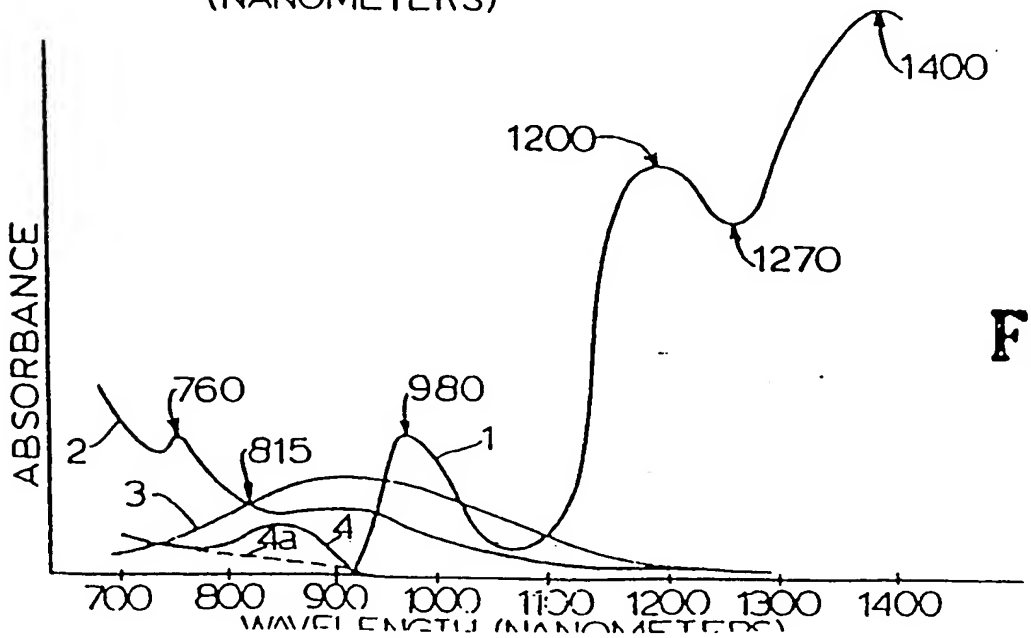


FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 88/03027

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL.(4) A61B 5/00 G01N 33/48

U.S. CL. 128/633 250/339 356/41, 320

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	128/632, 633, 664, 666. 250/339 356/40, 41, 320

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 3,790,797(STERNBERG ET AL)5 February 1974 Note Figure 1	
A	US, A, 4,299,487(SENGOKU ET AL)10 November 1981 Note Figure 1	
A	US, A, 4,281,645(JOBSIS)4 August 1981 Note Figure 6	
A	US, A, 4,655,225(DAHNE ET AL)7 April 1987 Note Figure 1	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

14 November 1988

Date of Mailing of this International Search Report

06 JAN 1989

International Searching Authority

ISA/US

Signature of Authorized Officer

William Wayner

W. Wayner